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HISTOCHEMICAL TECHNIQUES IDENTIFYING MAST CELLS IN PIG, CATTLE AND SHEEP*

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Abstract Carnoy's fluid and neutral buffered formalin (NBF) have been proved to be good fixatives for preservation of mast cells in pig, cattle and sheep except NBF blocked staining of most porcine mast cells, especially those located in intestinal mucosa (MMC) and in thymus medulla (TMMC). Both toluidine blue and Alcian blue were the excellent stains generally, but Alcian blue stained more porcine mast cells than did toluidine blue ($P<0.01$). Staining with toluidine blue of a wide pH range (from 0.1 to 7.0) showed that porcine mast cells were not very pH dependent, but the dye at pH 0.5 seemed to have the strongest affinity for all mast cells in pigs and it was also suitable for bovine and ovine mast cell staining. In the three species, unlike in rodents, the Alcian blue method did not distinguish between mast cells in the intestinal mucosa (MMC) and those in the connective tissue of the intestinal submucosa, tongue and skin (CTMC). Porcine CTMC, but not MMC, fluoresced strongly when stained with berberine sulphate or with a mixture of berberine sulphate and acridine orange. It suggested that porcine CTMC contained heparin proteoglycan.

Key words Mast cell, Histochemstry, Pig, Cattle, Sheep

Mast cells were originally identified in rat connective tissue by the numerous basophilic and metachromatic staining properties of their prominent cytoplasmic granules (Ehrlich, 1879) and it is known that toluidine blue, Alcian blue and berberine sulphate stainings are still the classic and ordinary methods for detecting mast cells in rodents and human (Jarrett *et al.*, 1984). We do not know very much about mast cells as well as mast cell histochemistry in some domestic animals.

More recently, the heterogeneity of mast cell populations has stimulated widespread interest and has become a focal point in discussion of mast cell biology (Kitamura, 1989; Galli, 1990; Wasserman, 1990; Gordon, 1990). The general concept now is that two distinct types of mast cells exist and these are termed the mucosal mast cell (MMC) and the connective tissue mast cell (CTMC). The two mast cell subpopulations can be distinguished by their histochemical, morphological and functional differences.

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Compared with CTMC, the staining properties of MMC differ in many aspects and are particularly sensitive to fixation and stainability to basic and metachromatic dyes (Enerback, 1966a, 1966b). The aim of this study was to evaluate the fixatives (Carnoy's fluid and formalin) and the dyes (toluidine blue, Alcian blue and berberine sulphate) in identifying mast cell proteoglycans in pig, cattle and sheep.

1 Materials and Methods

1.1 Animals and tissues

Tissue samples were obtained from five pigs (six month old), four calves (three week old) and five lambs (four month old) at slaughter. Samples of jejunum, tongue and thymus were collected from all animals and samples of skin, spleen, tonsil, lung and mesenteric lymph nodes were collected as required. One sample of each tissue was fixed by Carnoy's fluid (60% absolute alcohol, 30% chloroform and 10% glacial acetic acid) for 4 to 24 hours and another by 4% neutral buffered formalin (NBF) for 1 to 2 days. The tissues were embedded in paraffin. Sections were cut at 5 μm.

1.2 Histochemical stainings

1.2.1 Toluidine blue staining (Enerback, 1966a) Sections to be stained with toluidine blue were rinsed with 0.5 mol / L HCl (pH 0.5) for 5 min, stained with 0.5% (w / v) toluidine blue (Gurr, BDH, Poole, UK) in 0.5 mol / L HCl for 30 min, then washed with 0.5 mol HCl for 30 sec and counterstained with 0.25%(w / v) Safranin O in 0.125 mol / L HCl for 30 sec. Finally sections were washed with distilled water, blotted dry, brought rapidly through the alcohol series into xylene and mounted in DePex (Gurr, BDH, Poole, UK).

Toluidine blue (0.5%) in wide pH range (from 0.1 to 7.0) was also used to stain Carnoy-fixed pig tissues.

1.2.2 Alcian blue staining Sections were rinsed with 0.7 mol / L HCl (pH 0.2) for 5 min, stained with 0.5% w / v Alcian blue (Gurr, BDH, Poole, UK) in 0.7 mol / L HCl for 10 min (Spicer, 1960), washed with 0.7 mol / L HCl for 30 sec and counterstained with 0.25% Safranin O or light green.

Alcian blue was also used as a 0.1% (w / v) solution in 0.5 mol / L HCl (pH 0.5) with a staining time of 30 min (Wingren *et al.*, 1983) and followed by Safranin O or light green counterstaining.

1.2.3 Berberine sulphate staining (Enerback, 1974) Pig tissue sections were stained with a 0.02%(w / v) aqueous solution (pH 4.0) of berberine sulphate (Fluka AG, CH9470). The sections were mounted in glycerol, examined immediately using fluorescence microscopy (Leitz, Germany) and photographed. To compare the efficacy of berberine sulphate as a stain for porcine mast cells with that of toluidine blue, the sections stained with berberine sulphate were demounted with distilled water and restained with 0.5% toluidine blue (pH 0.5) for 5 min. Sections were dehydrated, cleared and mounted as mentioned above. The same fields as previously were re-photographed after toluidine blue staining.

A combined solution (1 : 1 v / v) of 0.02% berberine sulphate and 0.01% (w / v) acridine orange (Gurr, BDH, Pool, UK) was also used to stain pig sections and compared with berberine sulphate alone.

1.3 Image analysis and statistical analysis

Carnoy- or NBF-fixed and toluidine blue of Alcian blue stained sections of jejunum from 5 pigs, 4 calves and 5 lambs were examined using the field specific measurement program of the Optomax V (Analytical Measuring System, Cambridge, UK). To determine whether heterogeneity between MMC and CTMC, image analysis was used to compare the numbdr per mm^2 of MMC and / or CTMC stained with toluidine blue or Alcian blue in both Carnoy- or NBF-fixed tissues. Ten fields from jejunal lamina propria (MMC) and submucosa (CTMC) were compared for the animals. Student's t-test were performed to test the differences measured between and / or within groups of observations.

2 Results

2.1 Effects of fixatives and dyes

After fixation in Carnoy's fluid, CTMC in the gut submucosa, tongue, skin and the connective tissues elsewhere as well as MMC in the lamina propria of gut from the three species were strongly stained dark purple with toluidine blue (pH 0.5) (Figs.1, 3) and bright blue with Alcian blue (pH 0.2 or 0.5) (Fig.4). NBF was proved to be as good as Carnoy's fluid for the presevation of bovine and ovine mast cells (Fig.5), but NBF fixation obviously blocked the binding of the dyes to both porcine MMC and CTMC, especially MMC (Tables 1, 2) (Fig. 2). In NBF-fixed pig tissues, CTMC were weakly stained with or without surrounding metachromatic halos and MMC were usually faintly or diffusely stained or even completely unstained (Table 1, Fig. 2). Image analysis showed that the number of porcine mast cells per mm^2 varied with different fixatives and dyes. Compared with the results from the Carnoy-toluidine blue procedure, the NBF-toluidine blue procedure yielded far fewer

Table 1 Stainability of MMC and CTMC in porcine, bovine and ovine tissues in Carnoy and NBF fixed paraffin sections

Animal	Fixative	Mast cells	Alcian blue-Safranin O		Toluidine blue-Safranin O
			(pH 0.2)	(pH 0.5)	(pH 0.5)
Pig	Carnoy	MMC	B+++	B++++	P+++
		CTMC	B+++	B+++	P+++
	NBF	MMC	B+	B+	-
		CTMC	B++	B++	P+
Calf	Carnoy	MMC	B++++	B++++	P+++
		CTMC	B+++	B+++	P+++
	NBF	MMC	B+++	B+++	P+++
		CTMC	B+++	B+++	P+++
Sheep	Carnoy	MMC	B++++	B++++	P+++
		CTMC	B+++	B+++	P+++
	NBF	MMC	B+++	B+++	P+++
		CTMC	B+++	B+++	P+++

Remarks: Cytoplasmic granule colour: B = Blue; P = purple.

Staining intensity: -unstained; +weak; ++moderate; +++strong; ++++very strong

stained cells. 96.3 ± 3.4% of the MMC in number per mm^2 ($P<0.001$). While compared with Carnoy-fixed Alcian blue-stained tissues, 90.6 ± 2.5% of MMC in number per mm^2 ($P<0.001$) were blocked by NBF fixation. However, the submucosal CTMC in the same sections were partially preserved after NBF-fixation. Compared with what the Carnoy-fixed corresponding tissues had, NBF-toluidine blue procedure blocked 43.2 ± 26.7% of CTMC in the number per mm^2 ($P<0.001$) while NBF-Alcian blue 30.5 ± 23.6% ($P<0.005$).

Alcian blue stained significantly more porcine MMC ($P<0.01$) than toluidine blue when serial sections were stained at the same pH and time. The staining was unaffected by fixation procedures (Table 2). However, Alcian blue also stained the goblet cells in the intestinal epithelium making identification and counting of MMC difficult.

Table 2 The number of mast cells per mm^2 in sections of jejunum and thymus fixed in Carnoy's fluid and NBF and stained with toluidine blue and Alcian blue

Animal	Location of mast cells	The number of mast cells			
		Carnoy's fluid		NBF	
		Toluidine blue-Safranin O	Alcian blue-Safranin O	Toluidine blue-Safranin O	Alcian blue-Safranin O
Pig	MMC / Jej	274 ± 14 *	393 ± 10	11 ± 5	37 ± 3
	CTMC / Jej	265 ± 41	317 ± 38	141 ± 24	193 ± 6
	TMMC	255 ± 56	372 ± 92	72 ± 31	342 ± 73
Calf	MMC / Jej	298 ± 24	324 ± 27	NC	NC
	CTMC / Jej	50 ± 3	NC	NC	NC
	TMMC	507 ± 58	591 ± 43	NC	NC
Sheep	MMC / Jej	377 ± 14	NC	401 ± 20	NC

Abbreviations: NC = Not counted; Jej = Jejunum; * = The mean ± standard error.

Table 3 Intensity of staining of porcine mast cells in Carnoy's-fixed tissues with toluidine blue (0.5%) at different pH values

Type of mast cells	pH of 0.5% toluidine blus						
	0.1	0.3	0.5	1.0	3.0	5.0	7.0
MMC	P++—+++	P+++	P+++—++++	P++—+++	P++	RP+—++	RP—+
CTMC	P+++	P+++	P++++	P+++	P++—+++	RP+—++	RP+

Cytoplasmic granule colour: P = Purple; RP = Red-purple.

Staining intensity: -unstained; +weak; ++moderate; +++strong; ++++very strong.

2.2 Effect of pH on staining of porcine mast cells

Both porcine MMC and CTMC were well preserved in Carnoy-fixed tissues and were stained dark purple with toluidine blue at a pH range of 0.1 to 1.0. At less acidic pH values, however, the mast cell staining was weaker and redder, especially the MMC staining. The MMC staining was particularly sensitive to pH, although the cells could still be identified (Table 3). Toluidine blue at pH 0.5 seems to be the best stain for porcine mast cells and also for bovine and ovine mast cells.

2.3 Berberine sulphate binding

The granules in CTMC in pig tissues, including tongue, skin and intestinal

Fig.1 Mast cells in jejunal mucosa (MMC) and in the submucosa (CTMC) of a pig. Paraffin section of tissue fixed in Carnoy's fluid, stained with toluidine blue and counterstained with Safranin O. × 40

Fig.3 Mast cells in connective tissue (CTMC) of pig tongue. Paraffin section of tissue fixed in Carnoy's fluid, stained with toluidine blue and counterstained with light green. × 100

Fig.2 Paraffin section of a pig jejunum fixed in NBF, stained with toluididine blue and counterstained with Safranin O. Mast cells in the submucosa (CTMC) were stained and those in the mucosa (MMC) were not stained. × 40

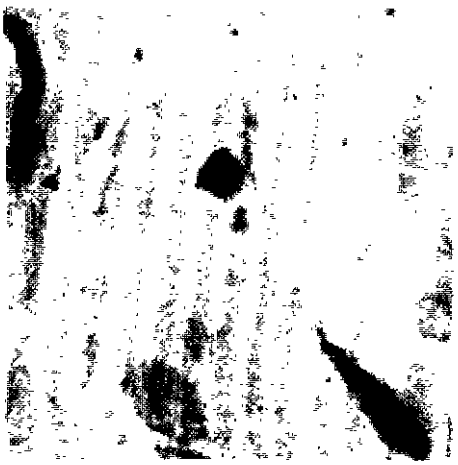

Fig.4 Mast cells in jejunal mucosa (MMC) of a pig. Paraffin section of tissue fixed in Carnoy's fluid, stained with Alcian blue and counterstained with light green. × 100

submucosa, fluoresced strongly yellow or bright orange-red when paraffin sections fixed in Carnoy's fluid were stained with berberine sulphate or the mixed solution of berberine

Fig.5 Mast cells in subcutaneous perivascular connective tissue (CTMC) of a calf. Paraffin section of tissue fixed in NBF, stained with Alcian blue and counteratained with light green. ×40

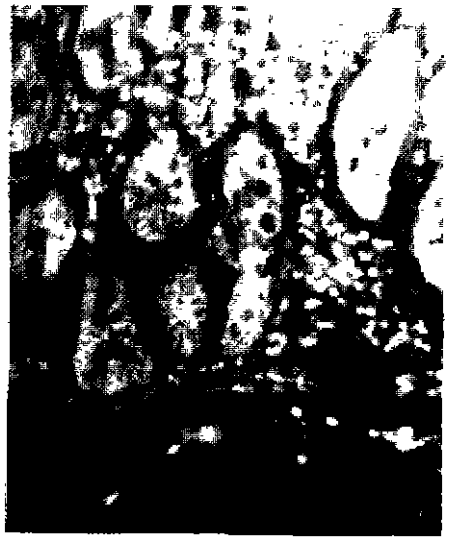

Fig.6 Paraffin section of pig jejunum fixed in Carnoy's fluid, stained with the mixed solution of berberine sulphate and acridine orange. Mast cells in the submucosa (CTMC) fluoreced bright orange-red and those in the mucosa (MMC) did not. ×40

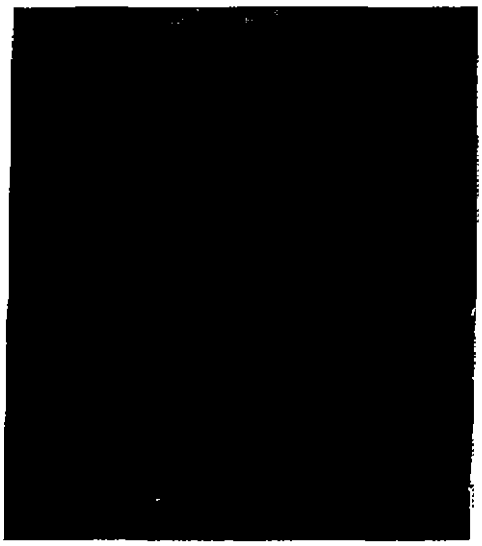

Fig.7 Paraffin section of pig tongue fixed in Carnoy's fluid, stained with the mixed solution of berberine sulphate and acridine orange. Mast cells (CTMC) in the tongue fluoresed bright orange-red. ×100

Fig.8 The same field of above section which was restained with toluidine blue conformed that the fluoresed cells were toluidine blue-positive mast cells.

sulphate and acridine orange (1 : 1 v/v) (Figs. 6, 7), whereas granules in jejunal mucosa (MMC) did not fluoresce (Figs.6). Identification of the fluoresced cells as CTMC was conformed by restaining the same sections with 0.5% toluidine blue (pH 0.5)(Figs. 7, 8).

NBF-fixation abolished the CTMC fluorescence with berberine sulphate staining and only weak orange-red fluorescence was observed in some CTMC (tongue and skin) after staining with the mixed berberine suphate-acridine orange solution.

3 Discussion

The topic of mast cell heterogeneity has recently been reviewed in detail by several authors (Kitamura, 1989; Galli, 1990; Gordon *et al.*, 1990). There are many evidences for both rodent and human mast cells expressing phenotypic variation in their histological and morphological characteristics. The phenotypical discrete mast cell populations may have different functions in health and disease.

It has been well documented that dye-binding of MMC is blocked by formaldehyde fixation. Staining of rat intestinal MMC was completely inhibited by routine fixation in 4% formaldehyde whereas CTMC were still stained (Enerback, 1966a). Similar results have been found for formaldehyde fixation in human MMC (Strobel *et al.*, 1981; Pipkorn *et al.*, 1988) and porcine mast cells (Xu *et al.*, 1993, 1994), although these cells were detectable with other fixatives, e. g. Carnoy's fluid, or with prolonged staining or trypsinisation (Enerback, 1987). Similarly, the dye-binding of MMC and CTMC in the three species could be well preserved with Carnoy's fluid fixation but porcine MMC staining was almost completely blocked by NBF fixation. $96.33 \pm 0.40\%$ by number per mm^2 ($P < 0.001$) were undetectable after NBF fixation compared with the corresponding toluidine blue-stained Carnoy-fixed specimens. In addition, porcine CTMC staining in the submucosa was considerably blocked by NBF fixation ($P < 0.05$). Bovine and ovine mast cells including MMC and CTMC, unlike those in rodents, human (Jarrett *et al.*, 1984) and pig (Xu *et al.*, 1993, 1994), could be well preserved by NBF fixation. Formaldehyde fixation has been found to block toluidine blue staining in 25%—30% of rat subepidermal mast cells and to reduce berberine sulphate binding in approximately half of the cells showed a weak fluorescence (Aldenborg *et al.*, 1988). Based on these evidences these authors considered these cells to be different from typical CTMC, or to be a subtype between MMC and CTMC.

These differences in histochemical reactions of mast cell granules have been attributed to differences of their granule proteoglycans which consist of a protein core and attached side chains of glycosaminoglycans, together accounting for two-thirds of the granular dry mass (Kitamura, 1989). Basic dyes forming ionic bonds with sulphated glycosaminoglycans in the granules made the mast cells detectable by light microscopy. Differences between MMC and CTMC in dye-binding phenotype and sensitivity to fixation may depend on the spatial arrangement, nature and properties of the glycosaminoglycan and protein in the granules. Sensitivity to fixation may be also related

to the cell maturity and lifespan. MMC in lamina propria have a short lifespan (< 40 days) may never reach maturity due to constant exposure to antigen attack and resulting degranulation, whereas CTMC (lifespan > 6 months) may shift finally toward maturity and formalin resistence (Jarrett *et al.*, 1984).

The present study showed that ① positive reaction with berberine sulphate was demonstrated in CTMC of porcine tongue, skin and intestinal submucosa, but not in MMC of intestinal mucosa at all. ② A double fluorescent staining of berberine sulphate and acridine orange could give a stronger fluorescent mast cells.

We did not try the berberine sulphate staining in bovine and ovine tissues. Berberine sulphate is a cationic fluorecent dye suitable for identification of CTMC in rodents because it form a strong fluorescent complex with the heparin proteoglycan (Enerback, 1974; Dimlich *et al.*, 1980). Rat MMC do not synthesize heparin proteoglycan, but chondroitin sulphate proteoglycan which fails to be binded by berberine sulphate. Unlike rodents and pig, both human MMC and CTMC contain heparin (Enerback, 1987) showing weakly positive in fluorescent berberine binding (Pipkorn *et al.*, 1988). Our study suggests that CTMC in pig, like in rat, contain heparin proteoglycan in their granules but MMC do not, so that fluorescent berberine binding can be used to identify porcine CTMC.

It was reported that rat mast cells reacted correspondingly with the pH level of fixative and the granules could be well preserved at acid pH levels but not pH 5–8 (Wingren *et al.*, 1983). This may be one reason why NBF fixation almost completely abolished the staining of MMC and reduced the stainability of CTMC. The different response to NBF fixation may also partially due to a loss of stainable materials through extraction by fixation (Enerback, 1966a) since the glycosaminoglycan of MMC with lower degree of sulpate (Tas *et al.*, 1977), appears to be more soluble than heparin of CTMC (Wingren *et al.*, 1983). Unlike rodent, humam and porcine mast cells, ovine mast cells and bovine lung mast cells (Chen *et al.*, 1990) could be well preserved by NBF fixation with toluidine blue staining.

It seems that not only the pH level of fixative can influence the preservation of mast cells, but also the pH levels of the staining solution. We noted that when the pH levels of 0.5% toluidine blue were below 3, good staining for both MMC and CTMC could be achieved in Carnoy-fixed tissues, but high pH levels made the mast cells become weaker and reddisher stained. Although pH3 (Ashraf *et al.*, 1988) and pH4.2 (Ceren, 1991) of toluidine blue has been used to identify porcine intestinal MMC, pH 0.5 of the dye seems give the strongest affinity for both MMC and CTMC in pig and also in cattle and sheep in our work.

Alcian blue, a copper phthalocyanin dye, has a larger molecular size with mW 1265 as compared to that of 306 for toluidine blue (Lillie, 1977) and has higher affinity to glycosaminoglycan of granules of MMC (Mayrhofer, 1980). Our work showed that fixation in Carnoy and followed by Alcian blue not only gave the very strong staining for both MMC and CTMC, but stained more porcine MMC than toluidine blue at the

same staining time and pH level. Even in the NBF-fixed tissues, more porcine MMC could be detected by Alcian blue. The disadvantage of Alcian blue stain might be that the goblet cells in the intestinal epithelinum would also be stained in blue and this might be a trouble for demonstrating or counting MMC.

In the present study, both MMC and CTMC in pig, cattle and sheep were stained in blue with Alcian blue and in purple with toluidine blue and on Safranin O-positive mast cells could be found in any Safranin O counterstaining. Similar results were reported in guinea-pig, human lung and bovine lung mast cells (Chen *et al.*, 1990). Safranin O counterstainings in this study, howerve, give a fine reddish background structures which form a better contrast with mast cells. Safranin O was commonly used as a counterstain for Alcian blue (Spicer, 1960; Combs *et al.*, 1965; Pipkorn *et al.*, 1988) and it could be used to distinguish MMC and CTMC in rodent (Enerback, 1966a, 1966b; Jarrett, *et al.*, 1984) with the MMC in blue and the mature CTMC in red. Regard to the results from non-rodent species with the Safranin O counterstaining, mast cell heterogeneity among species should be interpreted with great caution and further studied.

References

Aldenborg F, Enerback L, 1988. Histochemical heterogeneity of dermal mast cells in athymic and normal rats. *Histochem. J.*, **20**: 19-28.

Ashraf M, Urban J F Jr, Lee C M, 1988. Characterization of isolated porcine intestinal mucosal mast cells following infection with *Ascaris suum*. *Vet. Parasitol.*, **29**: 143-158.

Ceren M, 1991. Histometric study of mast cells in the duodenal wall of pigs. *Zbornik Vet Faku Univ Ljubljana*, **28**: 199-210.

Chen W, Alley M R, Manktelow B W *et al*, 1990. Mast cells in the bovine lower respiratory tract: morphology, density and distribution. *Br. Vet. J.*, **146**: 425-435.

Combs J M, Lagunoff D, Benditt E P, 1965. Differetiation and proliferation of embryonic mast cells of the rat. *J. Cell Biol.*, **25**: 577-592.

Dimlich R V M, Meineke H A, Reilly F D *et al*, 1980. The fluorescent staining of heparin in mast cells using berberine sulfate: Compatibility with paraformaldehyde or ophthalaldehyde induced-fluorescence and metachromasia. *Stain Technol.*, **55**: 217-223.

Ehrlich P, 1879. Contributions to the theory and practice of histological staining. In: Himmelweit F ed. The collected papers of paul ehrlich, vol.1. New York: Pergamon Press **1956**: 65-69.

Enerback L, 1966a. Mast cells in rat gastrointestinal mucosa, I Effects of fixation. *Acta Pathol. Microbiol. Scand.*, **66**: 289-302.

Enerback L, 1966b. Mast cells in rat gastrointestinal mucosa, II Dye-binding and metachromatic properties. *Acta Pathol. Microbiol Scand.*, **66**: 303-312.

Enerback L, 1974. Berberine sulphate binding to mast cell polyanions: A cytofluoremeteric method for the quantitation of heparin. *Histochem.*, **42**: 301-313.

Enerback L, 1987. Mucosal mast cells in the rat and human. *Intl. Arch. Allergy Appl. Immunol*, **82**: 249-255.

Galli S J, 1990. New insights into "The riddle of the mast cells" microenvironmental regulation of mast cell development and phenotypic heterogeneity. *lab Invest.*, **60**: 147-157

Gordon J R, Burd P R, Galli S J, 1990. Mast cells as a source of multifunctional cytokins. *Immunol. Today*, **11**: 458-464.

Jarrett E E E, Haig D M, 1984. Mucosal mast cells *in vivo* and *in vitro*. *Immunol. Today*, **5**: 115-119.

Kitamura Y, 1989. Heterogeneity of mast cells and phenotypic change between subpopulations. *Ann Rev. Immunol.*, **7**: 59-76.

Lillie R D, 1977 H J Conn's biological stains. 9 th eds Baltimore Williams & Wilkins. 221-225.

Mayrhofer G, 1980. Fixation and staining of granules in mucosal mast cells and intraepithelial lymphocytes in the rat jejunum, with special reference to the relationship between the acid glycosaminoglycans in the two cell types *Histochem. J.*, **12**: 513-526.

Pipkorn U, Karlsson G, Enerback L, 1988. Phenotypic expression of proteoglycan in mast cells of the nasal mucosa. *Histochem J.*, **20**: 519-525.

Spice S S, 1966. A correlative study of the histochemical properties of rodent acid mucopolysaccharides. *J. Histochem. Cytochem.*, **8**: 16-35

Strobel S, Miller H R P, Ferguson A, 1981. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques *J. Clin. Pathol.*, **34**: 851-858.

Tas J, Berndson R G, 1977. Does heparin occur in mucosal mast cells of the rat small intestine? *J. Histochem. Cytochem.*, **25**: 1058-1062

Wasserman S I, 1990. Mast cell biology. *J. Allergy Clin. Immunol.*, **86**: 590-593.

Wingren U, Enerback L, 1983. Mucosal mast cells of the rat intestine: a re-evaluation of fixation and staining properties, with special reference to protein blocking and solubility of thegranular glycosaminoglycan. *Hstochem. J.*, **15**: 571-582

Xu L R, Carr M M, Bland A P *et al*, 1993. Histochemistry and morphology of porcine mast cells. *Histochem. J.*, **25**: 516-522.

Xu L R, Carr M M, Bland A P *et al*, 1994. Histochemical and morphological heterogeneity amongst porcine mast cells. Proc of the 13th International Pig Veterinary Society Congress. Bangkok, Thailand 470.

鉴定猪、牛及绵羊肥大细胞的组织化学技术

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摘要 研究证实 Carnoy 液和中性缓冲福尔马林溶液（NBF）是猪、牛及绵羊肥大细胞的优良的固定液，NBF 虽能很好地保存牛和绵羊的肥大细胞，但却阻断了猪的大多数肥大细胞、特别是肠粘膜肥大细胞（MMC）及胸腺髓质肥大细胞（TMMC）对碱性染料的着染力。甲苯胺蓝及阿尔辛蓝均为动物肥大细胞的优良染料，但阿尔辛蓝能使更多的肥大细胞着染（$P<0.01$）。不同 pH 的甲苯胺蓝染色试验，显示猪肥大细胞对染液 pH 的要求虽不十分严格，但 pH 0.5 时似乎着染力最强，且也适用于牛及绵羊肥大细胞的染色。与啮齿动物不同的是，阿尔辛蓝染色并不能区分这 3 种动物的 MMC 及结缔组织肥大细胞（CTMC）。当采用硫酸小檗碱或硫酸小檗碱与吖啶橙混合液染色时，猪 CTMC 可显示出强荧光而 MMC 则无反应，证明猪 CTMC 含肝素蛋白多糖。

关键词 肥大细胞、组织化学，猪，牛，绵羊

中图分类号 Q26，S82